

# Retrogradation behaviors of damaged wheat starch with different water contents

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## ABSTRACT

The retrogradation behaviors of five damaged wheat starches (DS) after milling 0, 30, 60, 90, and 120 min with different water contents (33, 50, 60 %) were evaluated. Milling treatment increased DS content and developed an agglomeration of small particles. After 7 days of storage, the recrystallinity and long-range ordered structure of starch pastes were increased with the contents of DS and water. This process led to a lower setback viscosity and poor leaching of amylose. LF-NMR indicated a conversion from tightly bound water and free water to weakly bound water. During storage, DS12 with 60 % water content had the highest retrogradation tendency where the retrogradation enthalpy increased by 1.5 J/g and 2.2 J/g compared with DS0 with 60 % and DS12 with 33 % water content. DS with higher water content promoted the water mobility and made the starch molecular chains migrated conveniently. These changes facilitated the recrystallinity process during retrogradation period.

## 1. Introduction

Native wheat starch was a polymer macromolecule consisting of linear amylose and highly branched amylopectin, which had a semi-crystalline structure (Liu et al., 2019). When starch was heated with water, thermal energy broke the intermolecular bonds between starch molecules, exposing more hydroxyl sites to engage water, which led to a high swelling phenomenon and irreversible dissolution of starch granules in water. These changes referred to starch gelatinization without the birefringence of intact granules (Wang et al., 2015). During starch gelatinization, the structure of starch granules was destroyed with amylose leaching, resulting in a transformation from order to disorder status in starch granules (Pan et al., 2020).

When the gelatinized starch cooled down and stored at a certain temperature, amylose rearranged faster than amylopectin due to the linear structure. It required less space to develop a double helix or crystal than amylopectin. In contrast, amylopectin had a large number of branches, which got disordered after gelatinization and exhibited a slowly reorganization of ordered structure (Chang et al., 2021). Thus, amylopectin required a longer time for retrogradation than amylose. In addition, the process of starch retrogradation could be divided into two steps depending on the time of occurrence: the first process was a short-

term retrogradation process with order crystals developed by a double-helix structure from amylose through hydrogen bonding; the latter process referred to a long-term retrogradation process where an order double-helix structure was formed by the inter chain repolymerization of the amylopectin. The starch retrogradation depended on a number of factors, including water content, amylose/amylopectin ratio, molecular size, and chain-length distribution, etc (Zhu et al., 2020). In general, samples with higher content of amylose were prone to retrogradation. (Bresciani et al., 2022) reported that the higher the amylose content, the higher the hardness and the lower the stickiness of starch pastes. Higher content of amylopectin with longer chains generally formed more slowly digested starch during retrogradation (Martinez et al., 2018), thus indigestibility increased (Zhang et al., 2008). Albeit with no digestion analysis, (Matalanis et al., 2009) suggested that intramolecular and intermolecular associations were more prone to be formed by amylopectin with longer branches, which could increase the long-term retrogradation rate. In addition, during starch retrogradation, water was an important plasticizing agent for promoting the migration of starch molecules and contributed to the recrystallization processes. reported that the water content ranged from 20 to 90 % was suitable for starch recrystallization. Especially below 50 %, there was more available space for the mobility of starch chains, thus promoting the recrystallization of

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starch. When the water content exceeded 50 %, the cross-polymerization between starch chains occurred difficultly due to excessive dilution which was unbeneficial for the recrystallisation process.

Damaged starch (DS) commonly occurred during the milling process of cereal grains, which was accompanied by the disruption of granular structure and molecular degradation (Tran et al., 2011). Ball milling-induced mechanical forces resulted in a loss of starch integrity and a degradation of amylopectin into lower molecular weight fragments. it destroyed the hydrogen bonding between starch molecular chains and more hydroxyl groups exposed in the double-helix structure (Asmeda et al., 2016). This increased the binding ability of the starch to water molecules, thus promoting swelling and solubility (He et al., 2014), digestibility (Liu et al., 2020), and inhibiting the starch thermal transition and pasting viscosity (Bangar et al., 2023). However, few studies focused on the effect of starch retrogradation with different water content after milling treatment, which was unbeneficial for controlling the storage quality of starch-based products.

The current study aims to investigate the recrystallinity and water migration of starches with different water contents during retrogradation by differential scanning calorimetry, low field-nuclear magnetic resonance, X-ray diffraction, fourier transform infrared and rapid visco analyzer. Understanding the contribution of DS and water content as critical factors during starch retrogradation, could be useful for adjusting processing strategies and extending the shelf life of starchy foods.

## 2. Materials and method

### 2.1. Materials

Native wheat starch used in this study was from Ante Food Co., Ltd (Suzhou, Anhui, China). Other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

#### 2.1. Preparation of samples

Five damaged starch (DS) samples were obtained after milling 0, 30, 60, 90 and 120 min in a Planetary Ball Mill (Mitr Instrument Equipment, Changsha, China), which was named as DS0, DS3, DS6, DS9 and DS12, respectively. Their corresponding values of DS content were 0.68 %, 2.57 %, 4.22 %, 6.45 %, 7.45 % (Quinton & Kennedy, 2002).

#### 2.2. Particle size distribution

The particle size distribution of damaged starch samples were measured by a MS2000 laser particle size analyzer (Hifuta Technology, Beijing, China) (Asmeda et al., 2016).

#### 2.3. Scanning electron microscope (SEM)

The damaged starch samples were freeze-dried by a lyophilizer (Biocool Experimental Instrument, Beijing, China) before scanning electron microscope analysis (Carl Zeiss AG, Oberkochen, Germany). The magnification and accelerating voltage for SEM observation was set at  $\times 2500$  and 10.0 kV, respectively (Xie et al., 2019).

#### 2.4. Rapid visco analyzer (RVA)

Starch pasting properties were measured by FDV-E Rapid Visco Analyzer (Nirun, Shanghai, China) after being dispersed in water at a solid concentration of 8 % (w/w). Starch suspension was stirred with a paddle at 160 rpm until the end of the program (Zhang et al., 2017). The temperature procedure had been modified based on Chan et al. (2010). The demanded parameters included peak viscosity, through viscosity, breakdown viscosity, final viscosity, and setback viscosity were measured from viscometers.

### 2.5. Apparent amylose content (AAC)

During the RVA procedure, distilled water (20 mL) was added to the starch paste samples (10 mL) at the peak1, trough and peak2 viscosities, stirred well, and then centrifuged at  $4000 \times g$  for 5 min to determine the relative amount of amylose in the supernatant according to (Zhang et al., 2017).

As for the AAC values in retrogradation starch gels, damaged starch (0.8 g) and 30 mL of distilled water were heated at 100 °C for 30 min with constant stirring, and then stored in a refrigerator at 4 °C for 7 days, then took out and centrifuged at  $4000 \times g$  for 5 min to determine the relative amylose content in the supernatants, which was measured three times for each sample.

### 2.6. Differential scanning calorimetry (DSC)

Gelatinization and retrogradation characteristics of wheat starches were measured by a Q200 differential scanning calorimeter (TA Instruments, Delaware, USA). Starch (3 mg) were accurately weighted into an aluminum pan with distilled water to obtain different ratios of starch and water ratios in 1:0.5, 1:1 and 1:1.5 (w/v). The final water content arrived at 33, 50 and 60 %, respectively. All samples were equilibrated at 4 °C for 24 h before heating from 30 °C to 90 °C at a constant rate of 10 °C/min using nitrogen gas (50 mL/min). Subsequently, the gelatinized samples were stored at 4 °C for 7 days and then re-heated in the same profiles (Tao et al., 2021). The onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), conclusion temperature ( $T_c$ ), gelatinization enthalpy ( $\Delta H_G$ ) and retrogradation enthalpy ( $\Delta H_R$ ) of gelatinization were obtained by TA Rheology System Software (TA Instruments, Delaware, USA). Degree of retrogradation (%DR) was calculated according to the formula 1:

$$\%DR = \Delta H_R / \Delta H_G \times 100\%$$

### 2.7. X-ray diffraction

The crystal structure of starch particles was analyzed by a PANalytical X-Pert PRO MPD (Netherlands). The damaged or gelatinized starch samples were balanced in a sealed dryer with saturated NaCl solution for 7 days before being submitted to X-ray diffractometer. The sample was scanned from 5° to 35° at 2°/min under 40 mA and 40 kV conditions (Li et al., 2019). The relative crystallinity of starch samples was analyzed by Jade 6.0 software (Materials Data, California, USA).

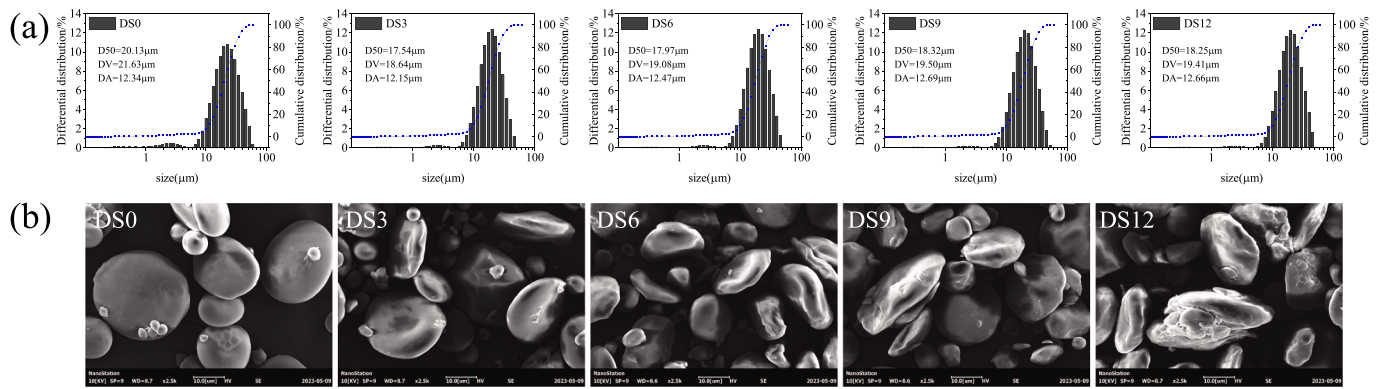
The retrograded samples for XRD were prepared by the method of Wang et al. (2016). Briefly, damaged starch samples (DS0, DS6, DS12) with water content at 33 %, 50 % and 60 % levels were equivalent at room temperature for 180 min, and then heated in a boiling water bath for 30 min with continuous stirring to obtain a complete gelatinization. Samples were cooled down slowly to room temperature and stored at 4 °C for 7 days. The retrograded samples were freeze-dried and milled for the recrystallinity measurement.

### 2.8. Fourier transform infrared (FTIR)

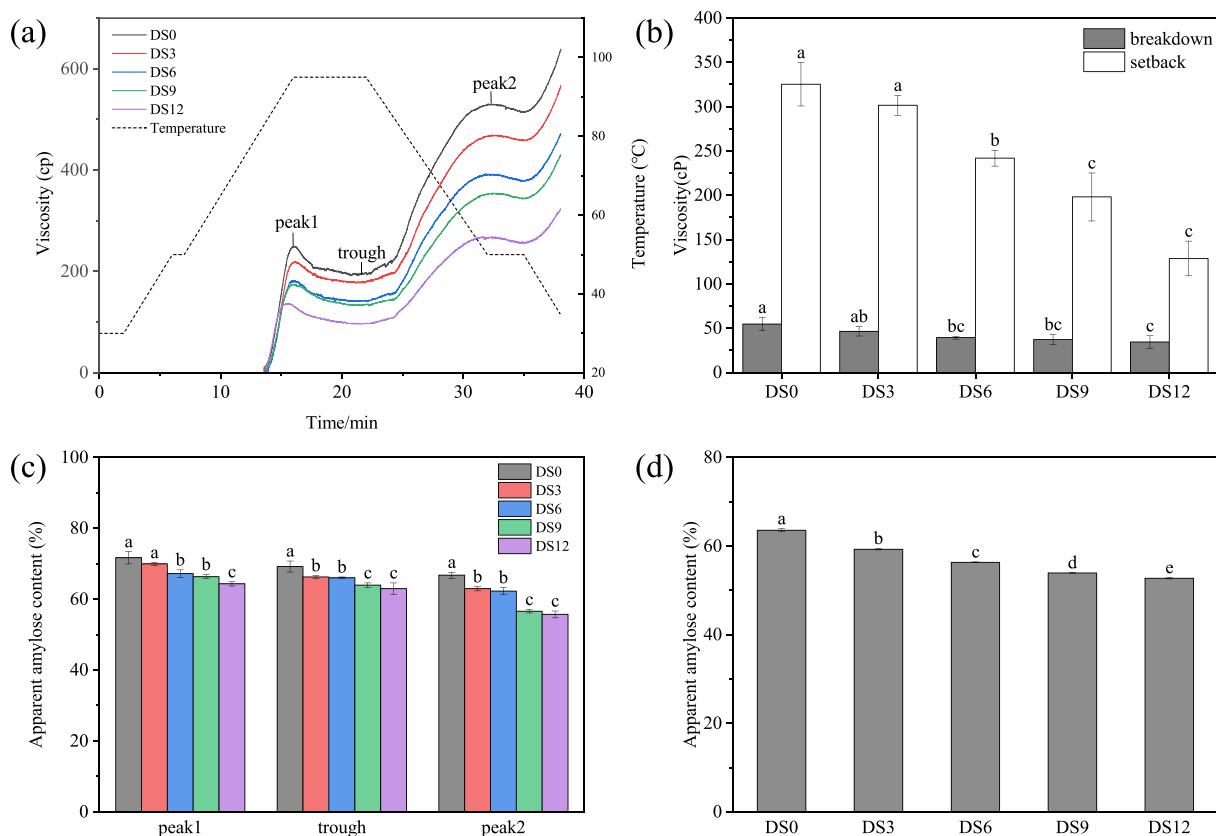
The short-range ordering structure of retrograded starch samples was analyzed by FT-IR spectrometer (Perkin Elmer Instruments, Massachusetts, USA) with 32 of accumulated scans (Li et al., 2019). The spectra from 600 to 4000  $\text{cm}^{-1}$  wavelength were collected at 4  $\text{cm}^{-1}$  of resolution before being subjected to OMNIC 8.0 software (Nicolet, Wisconsin, USA).

### 2.9. Low field-nuclear magnetic resonance (LF-NMR)

The water migration and distribution of all starch pastes after 7 days of storage were carried out with a low field-nuclear magnetic resonance (Niumag Electronic Technology, Suzhou, China) according to the



**Fig. 1.** The particle size (a) and SEM images  $\times 2500$  (b) of sample with different damage degree. D50, DV and DA represent medium diameter, volume average diameter and average surface area diameter, respectively. The bars indicate that the percent volume distributions of samples and the lines indicate that the frequency cumulative distribution curve of samples.



**Fig. 2.** RVA viscomogram (a) and pasting parameters (b) of sample with different damage degree and the relative amylose content of gelatinized (c) and retrogradation (d) starch.

method of (He et al., 2024) with some modifications. Samples were placed into a 2 mL vial and then inserted into the NMR sample tube. The parameters of test were as follows: RFD = 0.080 ms, NS = 16, SF = 18 MHz, SW = 200 kHz, TE = 0.30 ms, TW = 1000 ms, NECH = 3000.

## 2.10. Statistical analysis

The spectra curves were present with Origin 8.5 (Origin Lab Corporation, USA) while the statistics among all experiments were analyzed by SPSS16 (SPSS Inc., USA). with one-way of Variance (One-way ANOVA). Every experiment was conducted in at least three times and the significant differences ( $P < 0.05$ ) between groups were performed with Duncan test ( $P < 0.05$ ).

## 3. Results and discussion

### 3.1. Structural changes in damaged starch by ball milling

Fig. 1 showed the particle size distribution and SEM images of all starch samples. Native wheat starch was smooth and regular in shape. After ball milling for 30 min, the surface of starch granules underwent some deformation, exhibiting pronounced folds and more smaller granules (Soe et al., 2019), resulting in a left shift in the particle size distribution. After milling for 60 min, some granules were disintegrated, led to a formation of more irregular granule fragments. The starch granules were bonded to each other and became non-spherical. The mechanical force destroyed the interaction force between starch

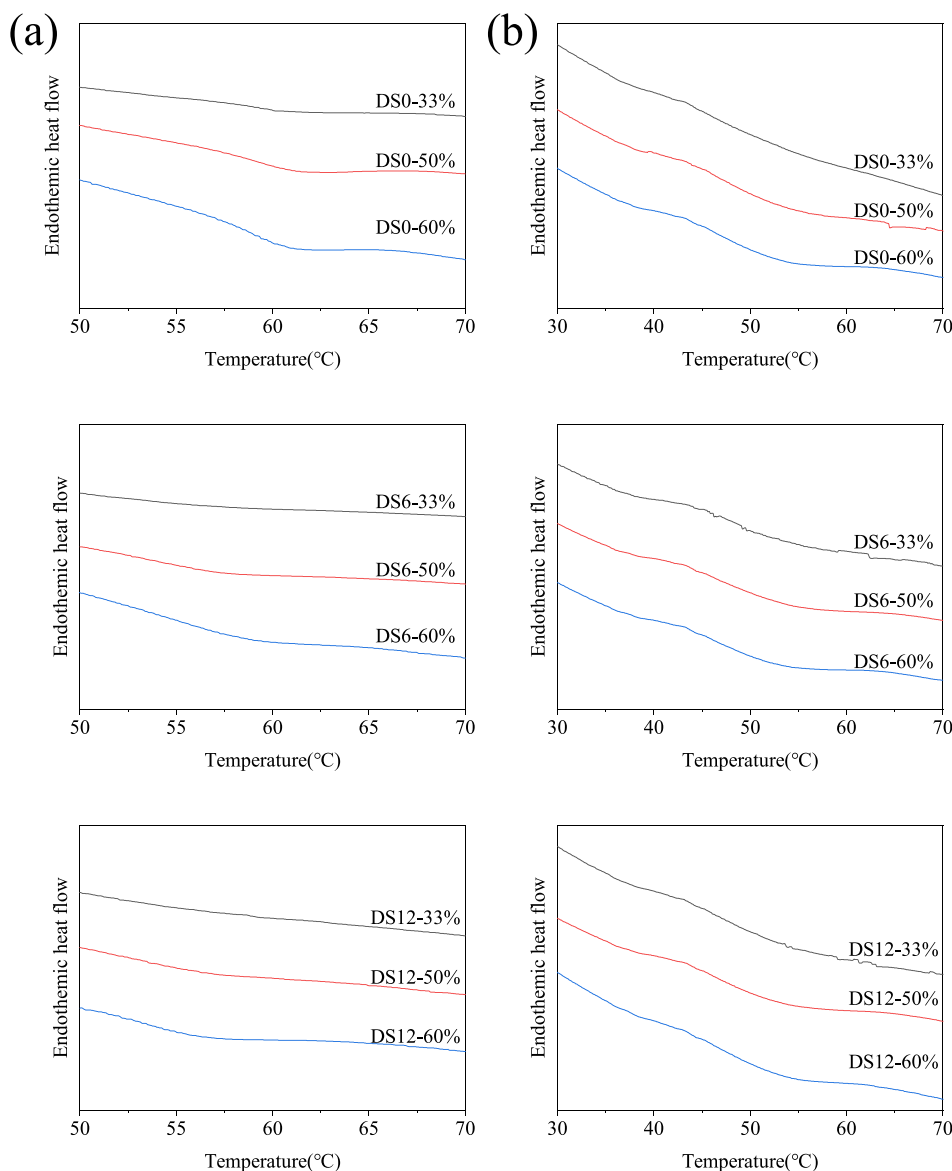


Fig. 3. DSC thermograms of gelatinization (a) and retrogradation (b) starch.

molecules, increased its specific surface area, and provided more surface free energy, thus contributing to an adhesion between disintegrated starch granules and developing a formation of agglomeration (Gonzalez et al., 2018). When the milling time increased beyond 60 min, there was a significant right shift observed in particle size distribution, indicating a higher agglomeration. Simultaneously, consistent trends were also observed in D50, DV and DA, although changes in DA were not significant. The D50 of native wheat starch were 20.13  $\mu\text{m}$ , reduced to 17.54  $\mu\text{m}$  after 30 min milling, and increased by 0.43  $\mu\text{m}$ , 0.78  $\mu\text{m}$  and 0.71  $\mu\text{m}$  respectively relative to DS3 when beyond 60 min milling.

### 3.2. Starch leaching during gelatinization and retrogradation

The pasting property of starch was an important indicator to reflect the starch quality. The RVA pasting curves and corresponding parameters of different damaged starch samples were presented in Fig. 2a and b, which showed a decreasing trend in the peak1, trough and peak2 viscosities with the DS increased. Similar decrement was observed in breakdown and setback values which was consistent with the published reports by Barrera et al. (2013). When starch granules were heated in water, the weak hydrogen bonds in the crystalline micelle region were

broken and contributed to higher capacity for hydration. It was accompanied by a disappearance of the crystalline region and a dissolution of amylose molecules. It increased the solution viscosity, which referred as peak1. After swelling process, starch viscosity gradually decreased because of the disrupted granular structure until it reached the trough viscosity. It indicated that the damaged granules have a higher swelling capacity with more free water inside and got more deformation under continuously shearing conditions. These changes were reflected as the higher values of breakdown viscosity in damaged starches. Ball milling treatment-induced weaker structure facilitated the disruption of the granules. During the cooling process, some starch molecules reassociated to form precipitated gel whereas the viscosity rose up to the peak2, these promoted the polymerization of leached amylose during colling period, named as setback. It depended on the leached amylose concentration and attractive forces between granules and continuous phase. The parameters of setback were reported to have significant correlation with the degree of polymerization (Chan et al., 2010). In order to define the dynamics of molecular leaching, rearrangement, and aggregation of starch during the heating and colling period, the relative amylose content at peak1, trough and peak2 were detected. As shown in Fig. 2c, the relative amylose content in the three

**Table 1**

The thermal properties of sample with different damage degree and water content.

| Samples   | $\Delta H_G$ (J/g) | $\Delta H_R$ (J/g) | %DR                  |
|-----------|--------------------|--------------------|----------------------|
| DS0-33 %  | $1.56 \pm 0.043^g$ | $1.82 \pm 0.009^j$ | $158.67 \pm 5.07^f$  |
| DS0-50 %  | $3.89 \pm 0.028^c$ | $4.35 \pm 0.031^g$ | $128.96 \pm 0.95^g$  |
| DS0-60 %  | $4.46 \pm 0.070^a$ | $5.26 \pm 0.033^d$ | $126.13 \pm 1.76^g$  |
| DS6-33 %  | $1.15 \pm 0.023^h$ | $3.56 \pm 0.018^h$ | $376.23 \pm 8.19^b$  |
| DS6-50 %  | $3.07 \pm 0.036^e$ | $5.02 \pm 0.015^e$ | $190.75 \pm 0.92^d$  |
| DS6-60 %  | $4.06 \pm 0.045^b$ | $6.44 \pm 0.029^b$ | $180.92 \pm 2.73^e$  |
| DS12-33 % | $1.04 \pm 0.020^i$ | $4.52 \pm 0.020^f$ | $615.79 \pm 12.16^a$ |
| DS12-50 % | $2.02 \pm 0.088^f$ | $6.24 \pm 0.013^c$ | $371.02 \pm 4.19^b$  |
| DS12-60 % | $3.54 \pm 0.021^d$ | $6.76 \pm 0.025^a$ | $276.78 \pm 4.18^c$  |

points was decreased with increasing damage degree, indicating that less amylose was leached out. Damaged starch was rich in an amylopectin continuous phase since their granular disintegration facilitated a diluted dispersion with a smaller occupied volume fraction. These characteristics caused a reduction in breakdown and setback in five damaged starch groups. The network of starch pastes was immersed with the swollen granules and fragmented starch granules. Furthermore, the relative content of amylose in the supernatant of starch pastes was further decreased after 7 days of storage (Fig. 2d). Because the gelation ordering and crystallization of amylose occurred in the earlier stages of starch retrogradation, less leached amylose were in the free state.

### 3.3. DSC

The DSC gelatinization curves of wheat starch were present in Fig. 3 and the corresponding parameters were shown in Table 1.. The results showed that  $\Delta H_G$  had a significant negative correlation with damage starch content, indicating less energy for crystalline disruption. Ball milling treatment destroyed the integrity of starch particles, and made the heating energy easily got through the starch particles, thereby reducing the thermal stability (Asmeda et al., 2016).

Starch retrogradation refers to a rearrangement and agglomeration of starch molecules by intramolecular hydrogen bonds in the cooling process. The enthalpy of retrogradation represents the amount of energy required to melt starch crystals during recrystallization. Table 1. showed the changes of retrogradation enthalpy in all starch samples. During storage, gelatinized starch underwent a rearrangement and agglomeration of starch molecules. After 7 days of storage, DS12 with 60 % water content had a highest value of  $\Delta H_R$  than others, indicating more gelatinized starch retrograded. The increased retrogradation enthalpy suggested that a more ideal crystalline structure emerged. It might be related to the breakage of the molecular chain caused by the ball milling treatment. Ball milling treatment disrupted the  $\alpha$ -1,6 glycosidic bonds in the amylopectin by breaking down the molecular chains which led to a

crystal formation of linear molecules. It resulted in a higher fluidity of linear molecules in starch pastes and accelerated the recrystallization process to form a higher degree of crystallinity. In addition, the disrupted structure of damaged starch also promoted water distribution and migration, which increased chain mobility and developed a formation of a nucleation and larger more ideal crystallites. Besides, the similar increased retrogradation trend was observed in the groups with different water contents. For example,  $\Delta H_R$  of DS0 was increased to 1.8, 4.3, and 5.2 J/g for 33 %, 50 %, and 60 % water content, respectively, indicating a better organized structure. The higher water content promoted the available space for the mobility of starch chains, thus contributing to the recrystallization process.

### 3.4. Crystal ordering of long and short term retrogradation

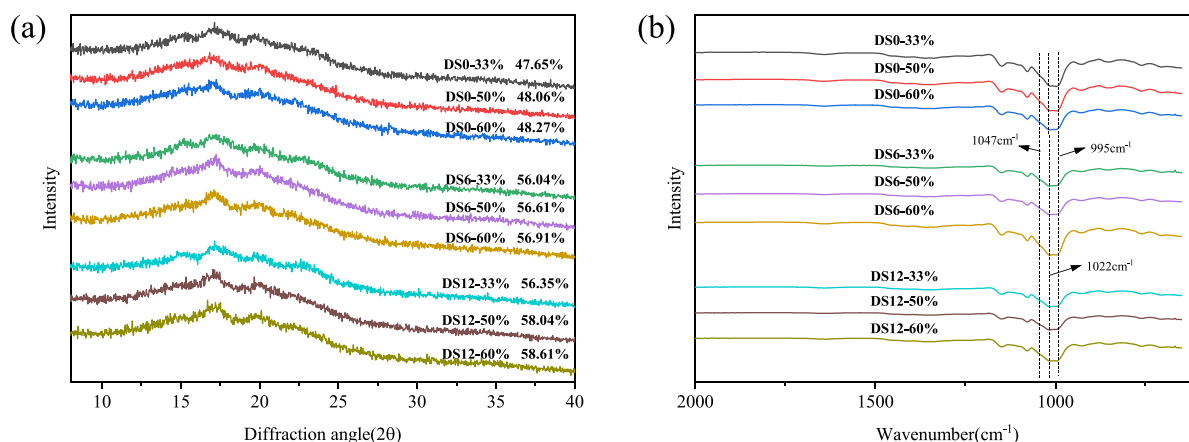
The structure of retrograded starch at a molecular level was analyzed by FT-IR and Fig. 4 showed typical absorption peaks at 1047, 1022 and 995  $\text{cm}^{-1}$ , which reflect the ordered, amorphous and water molecules regions, respectively (Lv et al., 2023). Thus, the absorbance ratios of 1047/1022  $\text{cm}^{-1}$  and 1022/995  $\text{cm}^{-1}$  represent the order degree of crystalline regions and the formation of double helices localized inside crystallites, respectively. They showed a positive correlation with the degree of short-range molecular order in starch samples. DS12 with 60 % water content had the highest ordering where the 1022/995  $\text{cm}^{-1}$  increased by 0.035 and 0.054 compared with DS0 with 60 % and DS12 with 33 % water content. Damaged starch with higher water content promoted the mobility of the starch molecular chains and made it recrystallized conveniently. As shown in Table 2., the 1047/1022  $\text{cm}^{-1}$  and 1022/995  $\text{cm}^{-1}$  values of damaged starch were generally higher than those of the DS0, indicating that higher DS and water content significantly increased the amount of recrystallisation from disorder to order status (Ma et al., 2021).

Retrogradation of starches resulted in the regeneration of the

**Table 2**

The long-range order of sample with different damage degree and water content.

| Samples   | IR ratio of 1047/1022 $\text{cm}^{-1}$ | IR ratio of 1022/995 $\text{cm}^{-1}$ |
|-----------|----------------------------------------|---------------------------------------|
| DS0-33 %  | $0.642 \pm 0.010^d$                    | $0.935 \pm 0.007^d$                   |
| DS0-50 %  | $0.658 \pm 0.001^{bc}$                 | $0.948 \pm 0.003^d$                   |
| DS0-60 %  | $0.667 \pm 0.002^a$                    | $0.996 \pm 0.004^{bc}$                |
| DS6-33 %  | $0.644 \pm 0.003^d$                    | $0.975 \pm 0.003^c$                   |
| DS6-50 %  | $0.664 \pm 0.002^{ab}$                 | $0.983 \pm 0.000^c$                   |
| DS6-60 %  | $0.654 \pm 0.001^c$                    | $0.994 \pm 0.003^{bc}$                |
| DS12-33 % | $0.652 \pm 0.007^c$                    | $0.977 \pm 0.012^c$                   |
| DS12-50 % | $0.654 \pm 0.001^c$                    | $1.005 \pm 0.005^b$                   |
| DS12-60 % | $0.669 \pm 0.001^a$                    | $1.031 \pm 0.030^a$                   |



**Fig. 4.** The XRD (a) and FTIR (b) pattern of retrogradation starch.

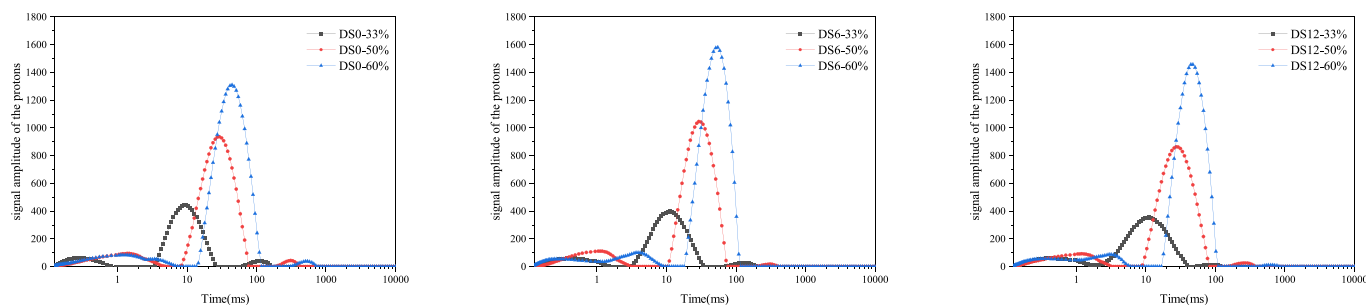


Fig. 5. Low field NMR spectra of retrogradation starch with different water content and degree of damage

Table 3

Relaxation curves of regenerated gels with different water content and degree of damage.

| Samples   | T <sub>21</sub> /ms      | A <sub>21</sub> /%          | T <sub>22</sub> /ms       | A <sub>22</sub> /%          | T <sub>23</sub> /ms          | A <sub>23</sub> /%         |
|-----------|--------------------------|-----------------------------|---------------------------|-----------------------------|------------------------------|----------------------------|
| DS0-33 %  | 0.49 ± 0.07 <sup>d</sup> | 12.43 ± 1.73 <sup>abc</sup> | 9.23 ± 0.37 <sup>e</sup>  | 82.84 ± 1.28 <sup>f</sup>   | 121.11 ± 4.40 <sup>f</sup>   | 4.47 ± 0.35 <sup>a</sup>   |
| DS0-50 %  | 1.23 ± 0.27 <sup>c</sup> | 11.94 ± 0.31 <sup>bcd</sup> | 28.68 ± 1.14 <sup>d</sup> | 86.61 ± 0.33 <sup>d</sup>   | 287.08 ± 15.35 <sup>e</sup>  | 1.45 ± 0.08 <sup>cd</sup>  |
| DS0-60 %  | 3.04 ± 0.33 <sup>b</sup> | 10.40 ± 0.27 <sup>e</sup>   | 44.49 ± 0.00 <sup>b</sup> | 88.92 ± 0.32 <sup>abc</sup> | 1349.47 ± 19.22 <sup>a</sup> | 0.79 ± 0.26 <sup>d</sup>   |
| DS6-33 %  | 0.68 ± 0.06 <sup>d</sup> | 13.49 ± 1.19 <sup>ab</sup>  | 11.40 ± 1.23 <sup>e</sup> | 83.41 ± 0.12 <sup>ef</sup>  | 129.26 ± 10.11 <sup>f</sup>  | 2.78 ± 1.32 <sup>b</sup>   |
| DS6-50 %  | 1.12 ± 0.08 <sup>c</sup> | 10.83 ± 0.30 <sup>cde</sup> | 32.30 ± 3.48 <sup>c</sup> | 88.20 ± 0.25 <sup>bc</sup>  | 334.91 ± 21.57 <sup>d</sup>  | 0.97 ± 0.43 <sup>cde</sup> |
| DS6-60 %  | 3.49 ± 0.14 <sup>a</sup> | 10.00 ± 0.34 <sup>e</sup>   | 48.83 ± 1.98 <sup>a</sup> | 89.89 ± 0.35 <sup>a</sup>   | 936.34 ± 12.91 <sup>c</sup>  | 0.10 ± 0.02 <sup>e</sup>   |
| DS12-33 % | 0.73 ± 0.08 <sup>d</sup> | 13.72 ± 1.57 <sup>a</sup>   | 11.40 ± 1.23 <sup>e</sup> | 84.48 ± 1.87 <sup>e</sup>   | 132.55 ± 5.67 <sup>f</sup>   | 1.78 ± 0.48 <sup>c</sup>   |
| DS12-50 % | 1.15 ± 0.09 <sup>c</sup> | 11.30 ± 0.21 <sup>cde</sup> | 28.68 ± 1.14 <sup>d</sup> | 87.57 ± 0.47 <sup>cd</sup>  | 258.81 ± 20.25 <sup>e</sup>  | 1.13 ± 0.28 <sup>cd</sup>  |
| DS12-60 % | 3.33 ± 0.13 <sup>a</sup> | 10.65 ± 0.16 <sup>de</sup>  | 47.69 ± 0.00 <sup>a</sup> | 89.24 ± 0.09 <sup>ab</sup>  | 1259.42 ± 34.23 <sup>b</sup> | 0.39 ± 0.14 <sup>e</sup>   |

crystalline structure, which was reflected as high intensity of major crystalline peaks at 15°, 17°, 23° and 20° (Fig. 4a). The gelatinized starch showed the apparent peaks at 15°, 17° and 23° means a typical A-type crystalline structure, while appeared a V-type crystalline during storage. The occurrence of diffraction peaks at 20° was mainly attributed to the formation of amylose with the endogenous lipids (Chao et al., 2018). These relatively distinct peaks revealed a dynamic alignment and reorganization in the gelatinized starch during retrogradation, thus developing a number of double helices as well as some microcrystalline structures. Compared with the DS0 sample with 60 %, ball milling treatment significantly increased the relative crystallinity of from 48.27 % to 56.91 % and 58.61 % respective. Ball milling treatment disrupted the α-1,6 glycosidic bonds in the amylopectin by breaking down the molecular chains which led to a crystal formation of linear molecules. It resulted in a higher fluidity of linear molecules in starch pastes and accelerated the recrystallization process to form a higher degree of crystallinity. More ideal crystalline need more retrogradation enthalpy to form, which was consistent with the conclusion obtained in DSC analysis. Besides, the disruption of hydrogen bonding interactions between damaged starch molecular chains led to a partial unspinning of the double helix structure and produced more free amylose and amylopectin. During the retrogradation, the molecular chains of these

free amylose and amylopectin were rearranged to form crystals by new hydrogen bonding, which was essentially a result of the ordered arrangement of molecular chains. In addition, different water contents changed the relative crystallinity with the increased intensities at 17° and 20°. It indicated that water molecules promoted the formation of crystalline phases during the retrogradation process due to the higher mobility of starch chains inside granules. The cross-polymerization between starch chains occurred easily which was beneficial to the rearrangement of crystalline regions.

### 3.5. LF-NMR

LF-NMR was a necessary tool to determine the status of water in the retrograded starch. Fig. 5 showed three distinct Carr-Purcell-Meiboom-Gill (CPMG) signal peaks (T<sub>21</sub>, T<sub>22</sub> and T<sub>23</sub>) in the decay relaxation curves and their corresponding areas were reflected as A<sub>21</sub>, A<sub>22</sub> and A<sub>23</sub> (He et al., 2023). They were individually related to the tightly bound water, weakly bound water and free water (Zhou et al., 2022). The high level of water promoted the relaxation curves of all retrograded starch samples shifted significantly to the right, where the A<sub>22</sub> showed an increased range from 82.84 % to 89.89 % in Table 3.. It suggested that the tightly bound and free water were converted to weakly bound water during retrogradation. It could be attributed to the more short linear chains in damaged starch, which has been confirmed by Chen et al. (2019). At the low water content (33 %), damaged starch exhibited a decrease of free water and the tightly bound water and weakly bound water have an opposite trend. At higher water contents (50 % and 60 %), the tightly bound and free water were transformed into weakly bound water as the degree of DS increased. During ball milling, the structure of starch granules was disrupted and hydrogen bonds were broken to some extent, resulting in an increase of the ability of starch to bind with water molecules, which limited water molecules' capacity to entrap in the sample. Moreover, after ball milling for 120 min, starch granule appeared severe breakage and resulted in a looser structure, thus providing more opportunity for the conversion of bound water into free water, which was entrapped in the starch crystals.

## 4. Conclusions

This study investigated the contribution of damaged starch level and water content to starch retrogradation and showed a remarkable recrystallinity in all damaged starch group at 33–60 % of water content. The effect of water content on the recrystalline structure and water migration in different damaged starches was shown in this study. Ball milling treatment resulted in a breakage of the molecular chain of the starch samples by disrupting the α-1,6 glycosidic bond, facilitating higher fluidity of the linear chains and promoting the cross-linking behavior for recrystallization. Meanwhile, DS resulted in a limitation for amylose leaching increased and more amylose molecules recrystallized as an ordered structure. Damaged granular structure allowed more water absorbed, and was beneficial to the transformation of the order chains of amylose and amylopectin into the packed double helices and

crystalline.

## CRediT authorship contribution statement

**Han Tao:** Writing – original draft, Funding acquisition. **Xiao-Han Fang:** Writing – original draft, Investigation. **Wan-Hao Cai:** Writing – review & editing. **Song Zhang:** Supervision, Investigation. **Hui-Li Wang:** Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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